Genome editing with modularly assembled zinc-finger nucleases

To the Editor: In a Correspondence in *Nature Methods*, some members of the Zinc Finger Consortium reported discouragingly high failure rates for the modular assembly of zinc-finger DNA-binding proteins and concluded that more time-consuming and labor-intensive selection-based methods were "the only publicly available alternatives for academic researchers interested in using ZFN technology"[1]. Zinc finger nucleases (ZFNs) are artificial restriction enzymes made by fusing reprogrammable zinc-finger DNA-binding units to the FokI nuclease domain, which efficiently induce, site-specific mutations in higher eukaryotic cells, and thus hold great promise in many fields. Three methods have been developed to make ZFNs: a proprietary method used by Sangamo Biosciences, the modular-assembly method via standard recombinant DNA technology[2] and cell-based selection methods[3] (**Supplementary Fig. 1**). Each of these methods has pros and cons, and we wish to inform potential ZFN users of them.

Contrary to the conclusion in Ramirez *et al.*[1], recent reports by three groups led by us[4–6] and the Zinc Finger Consortium members themselves[7] demonstrated that modularly assembled ZFNs had genuine potential for genome editing in several experimental systems. Tests in plant cells of the genome-editing activities of modularly assembled ZFNs and those produced via an *Escherichia coli*–based selection method, termed oligomerized pool engineering (OPEN), showed that modularly assembled ZFNs not only induced site-specific mutations in the plant genome but also outperformed ZFNs made using OPEN in terms of mutation frequencies[7]. The overall success rates with the two methods were comparable; each resulted in genome modification at one out of four target sites. We recently reported that modularly assembled ZFNs induced targeted mutations in the human genome[4]. The success rate of the modular-assembly approach using publicly available resources was 24%, meaning that almost one out of four target sites could be modified. (Out of 315 ZFNs we tested at 33 different genomic sites in human cells, 21 ZFNs showed successful genome-editing activities at 8 sites.) Furthermore, we found that modularly assembled ZFNs could induce large chromosomal deletions in human cells[5], and we also used modular assembly to produce ZFNs that induced targeted mutagenesis in *Drosophila melanogaster*[6].

We summarized all the ZFNs reported in the literature that gave rise to site-specific mutations at endogenous loci in higher eukaryotic cells and organisms (**Supplementary Table 1**). Out of 57 ZFNs that showed genome-editing activity, 23 had been modularly assembled using publicly available zinc fingers, and 15 were constructed via selection methods. The remaining ZFNs were constructed by Sangamo Biosciences, Inc., using a proprietary archive of zinc-finger modules.

It is possible that selection methods may take the context effect of neighboring zinc fingers into account and yield more reliable and effective zinc-finger arrays than does modular assembly[3]. However, these selection methods are highly labor-intensive and time-consuming. Thus, only a limited number of ZFNs can be prepared in parallel, a process that still takes at least several weeks, if not months. In contrast, the modular-assembly method allows construction of hundreds of ZFNs in a few weeks using standard recombinant DNA technology. Furthermore, ZFNs made using OPEN thus far are largely limited to targeting GNN repeat sequences, where G is guanine and N is any base (that is, 5′-GNNGNNGNN-3′), which occur rarely in a given gene of interest. Ramirez *et al.*[1] also reported high failure rates of modularly assembled ZFNs at non-GNN repeat sites and 100% failure rate at sites free of the GNN motif. We note, however, that both Sangamo and ToolGen have achieved success without this bias, even with sites completely lacking the GNN motif[4,8].

Ramirez *et al.*[1] duly raised concerns about the high failure rates they observed with modularly assembled ZFNs, but we suggest that researchers interested in ZFN technology should not be discouraged from using the fast, easy-to-practice modular-assembly method and that there is a need for further studies using this method. We expect that the modular-assembly method will be improved when used by many scientists. For example, we found that not all zinc fingers were equally effective as modules for making functional ZFNs and that careful choice and use of reliable modules could improve success rates[4]. We believe that further studies testing diverse zinc fingers will lead to an understanding of capabilities and limitations of ZFNs and to an appreciation of the best approaches for ZFN design.

Note: Supplementary information is available on the Nature Methods website.

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COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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Joung *et al.* reply: The publications cited by Kim *et al.*[1] describing successful construction of zinc-finger nucleases (ZFNs) by modular assembly only further support our original conclusion that this method has a high failure rate for engineering functional zinc-finger arrays[2]. Two[3,4] of the three reports cited